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December 17, 1968

Dr. Marshall Nirenberg
Chief, Section on Biochemical Genetics
Laboratory of Clinical Biochemistry
National Heart Institute
Building 10, Room 7D-03
National Institutes of Health
Bethesda, Maryland 20014

Dear Marshall:

Here is a transcription of my notes of a conversation with Dr. Brenner. I'm sorry that the press of work delayed my completion of it. And even I had trouble reading my scribbled notes.

The transcription also includes Dr. Benzer's recent comments on his work. Together, I hope they are useful to you in connection with the remarks or report that will kick off the last day of the next Stated Meeting. I'm sorry neither Brenner nor Benzer could attend. But then you have that much less introducing to do as chairman.

Also enclosed are our Polaroid copies of the slides shown in conjunction with Dr. Gajdusek's talk at an earlier Stated Meeting.

Under separate cover I am sending a copy of the tape recordings that include Dr. Gajdusek's presentation at an earlier Stated Meeting. Wardy Holman included in his package of the two tapes the track and foot count of the start and finish of the presentation.

With best Holiday wishes, I am

Sincerely,

Ted Melnechuk

Theodore Melnechuk
Director of Communications

Enclosures: 2

PRIVILEGED COMMUNICATION

T. Melnechuk
December 16, 1968

Notes of a Conversation on the Morning of October 30, 1968 at the NRP Center Between Professor F.O. Schmitt and T. Melnechuk and guest, Dr. Sydney Brenner of the Medical Research Council Laboratory of Molecular Biology at Cambridge, England

I. BACKGROUND

At the Stated Meeting of NRP Associates in February 1968, Dr. Marshall Nirenberg gave a brief account of the research strategy he thought he might follow in studying the behavior genetics of simple neural systems. Believing it would be interesting to hear similar accounts from two other eminent molecular geneticists, Drs. Seymour Benzer and Dr. Sydney Brenner, Professor Schmitt invited the latter two investigators to speak at the Stated Meeting in February 1969, with Dr. Nirenberg chairing. Neither invitee could be present at that time, but each responded with an account of their strategy, and Dr. Brenner included a status report as well.

Dr. Benzer's account was in the form of a paragraph in a letter to Professor Schmitt dated December 3, 1968. I cite the paragraph in full:

> The work my group and I are engaged in is the isolation of behavioral mutants of Drosophila, particularly in respect to the phototactic response, with the idea

> of obtaining genetic blocks at the various steps involved. We look at the mutants for anatomical or developmental defects affecting the nervous system and also at electrical events in the visual system, as well as biochemical aberrations. Much of the work is still very preliminary, but we have begun to find interesting electroretinogram abnormalities in certain visual mutants and are writing up that work for publication. I will send you a copy of the paper when it is ready, for your information.

It is also possible that Dr. Benzer will describe his neurobiological work at a symposium on "Control Processes in Multicellular Organisms" scheduled to be held in New Delhi next March 16-20 by The Ciba Foundation. In a preliminary program dated March 14, 1968, Dr. Wolstenholme has Dr. Benzer slated to speak on "Genetics of Brain Function" at 4:30-5:00 on Thursday, March 20, 1969.

Dr. Brenner's comments were made in the course of a two-hour conversation with F. O. Schmitt and T. Melnechuk on October 30, 1968. Before reporting that conversation, however, it seems helpful to abstract the main points made by Dr. Brenner two days earlier at the Massachusetts General Hospital in giving the Warren Triennial Prize lecture.

II. "THE FUTURE OF MOLECULAR BIOLOGY"

Under the title just given, Dr. Brenner made several major points:

1. Scientists tackle problems that they consider important. Of these, they tackle those problems they consider solvable. And problems become solvable through the onset of new techniques, or new conceptions, or both.

2. Current major problems include the physical mechanisms of:

   a. Polypeptide folding

   b. Enzyme action

   c. Protein repression and induction

3. The nature of a scientific explanation is different in physics and biology. In physics, an explanation is ultimately a differential equation. But in biology, an explanation is ultimately a list--for example, a program.

4. Only Max Delbrück expected that molecular biology might discover new principles of physics, according to Gunther Stent's "That Was the Molecular Biology That Was" (Science, Vol. 160, pages 390-395, 26 April 1968; a copy is attached as an appendix).

5. The goal of molecular biology is to be able to compute an organism from a knowledge of its genes.

6. The Monod-Jacob notion of repression-and-induction is a sufficient key to understanding development.

7. Just as "Project K"--to know all about the genetics of the biochemistry of E. Coli--is feasible, so is "Project Man," about whose physiology much is known.

8. In order to yield as complicated and hierarchically arranged an organism as man, a genetic program must surely have parts, so there is some hope of getting at its logic before getting a thorough knowledge of its details. By "part of a program" is meant such a sub-program, focused on an organ or a function or constituents, as would permit the eye to evolve without concomitant evolution of the kidney.

9. Molecular Biology is essentially the Chemistry of Natural Selection, so it must eventually deal with the interplay of the genetic program with the accidents of history.

III. CORRELATIVE NEUROBIOLOGY

Unfortunately, Dr. Brenner's remarks at the NRP were not tape-recorded. What follows is an edited transcription of T. Melnechuk's notes, which were not made with this eventual use in mind:

Staff.-

Drs. Brenner and Crick now have a staff of 14 scientists. After a building delay, they will have 28, who will work in three

groups, one on molecular genetics, one on cell biology, and the largest on neurobiology. The technical staff includes technicians trained to photograph anything at scales from the visible down to the EM level.

Subject.-

Brenner's own work is in neurobiology: not so much molecular--that is, not on membranes, and not on biochemistry, except where relevant to the genetic problem,--as on the genetic control and specification of structures in the nervous system: what they are and how they got there. He wants to know what single genes can do, both structurally and behaviorally.

Organism.-

He prefers to work at this time on a system with no learning, one that is rigorously specified, controlled from within. Therefore, he does not work with goldfish, but with nematodes.

The small nematode he works with most is 500-700 mu long in "adolescence" and 1 mm long when adult. The specimens are "identical", being naturally inbred, for in their 3-day life cycle, each nematode begets 300, so that in a week, one becomes 10^5, all on one Petri dish.

Neuroanatomy.--The small nematode probably has less than 500 neurons. Brenner is cutting serial sections 10 mu thick,

using EM with 30 A resolution. The job is being done broadly first, to get technical facility. The entire sensory system has been defined, the neurons counted, and the dendrites pictured, but the hook-up of the sensory system to the central nervous system is not yet known. All the sensory neurons have dendrites like cilia, with microtubules.

Work continues on the motor side. Work on the innervation of the esophagus is half done. He has identified 92 muscle cells, though not what these cells do. Each set of 16 muscle cells constitutes a ring. Such muscle cells modulate like neurons. They have oblique striations, rather than sarcomeres.

The nematode's head, which narrows down, receives projections from 121 cells. To get a three-dimensional grasp of the neural processes, Brenner prefers getting a mental picture by handling the actual cross-sectional EM pictures. Neither a cinemicrotome approach a la Livingston nor an analog of the X-ray crystallographer's overlay method work for him; he wants to reconstruct the nervous system in his own mind.

Behavior.- Nematodes have a limited behavioral repertoire. It is difficult to describe their behavior scientifically. Brenner takes cine pictures and does a frame-by-frame analysis.

Of the 200 mutants he has observed, most are behavioral--usually forms of paralysis. He picks the mutants by a brute-force

method: he examines a colony, and picks up the cripples with a toothpick.

Genetics.-Brenner is doing an extensive genetic analysis. The behavioral alterations are produced by mutations of 22 easily recognizable genes; this number will go up to about 30. The genetic map is saturated; 1 gene has 8 mutants. The X chomosome is mapped, and the autosomes identified as linkage groups. This work will take 2-3 months more to finish.

The next step is to identify the structural defect in each mutant, so that if there is a change, it can be seen. This will give some idea of what single genes can do, both structurally and behaviorally.

Transmitter Biochemistry.- To do the biochemistry of nematode transmitters, large numbers of animals are needed, so that mass culture techniques are necessary. Work is almost ready to begin. The idea is that if one is defective, you have "the edge of the wedge". For example, you could study what the behavior would be if there were no inhibitory transmitter--which, by the way, GABA is suspected to be in this organism.

Brenner named three people who will be working on nematode neurochemistry: Tony Stratton or Stretton, who was with Vern Ingram; Slater or Slayter, and Dennis Bray, both of whom have been with Steve Kuffler.

Neurophysiology.- Unfortunately, the particular animal chosen for being appropriate to genetics and electron microscopy is not appropriate for neurophysiological studies. They are too small for the insertion of electrodes.

However, large nematodes exist: the ascarids. Brenner believes that their basic structure is similar enough to that of the small nematodes so that the only difference is one of scaling up the 5 or 6 levels from the free-living nematodes. He says that Goldschmidt's old anatomical work is mostly wrong, by the way.

Because of their complicated parasitical life cycle, ascarids can't be grown in the laboratory. But neurophysiological studies can be performed, and the findings presumed transferable to the smaller species.

Philosophy.- Brenner said he was not for, indeed was against, the idea of a changeable genome. Instead, he was for answering the question: Given the wiring diagram, what else must be known in order to be able to compute the organism's behavior?

Next, a deeper problem: How is it constructed? The accurate computation of structure in development argues an utterly deterministic, digital program. The specificity and precision of detail could not result from analog methods, such as gradients of concentration. But only the genes are digital. There must, there-

fore, be accurate gene switching.

But the interplay of the long phylogenetic and ontogenetic process with history and its accidents during the prolonged development of elaborate organisms argues that such complicated systems must be subject to additional, new constraints, such as economy and continuity in evolution (as in the case of the persistence of cilia in sensory receptors). Complicated systems may, therefore, employ analog principles as well as the digital principles of simpler systems.

Brenner believes that storage is not in molecules but in cell assemblies, by means of synaptic switching. Given the switches, their logic, and the plasticity of the complicated nervous system, he wants to know how it works. He sees the need of an analysis of function space.

IV. OTHER LINES

Permeability.- Brenner considers this question too wide for him.

Molecular Biology of Transmitters.- Here, Brenner would like to see the protein receptor molecules isolated, and has two chemists coming to work at this problem in a professional manner. He wants to work on Miledi's preparation, denervating a muscle and watching the spread of sensitivity. He thinks too many people are already working on Torpedo.

In his terms, he is after "the machine language of learning." Of the receptor molecule species he would like to know: Is it localized? If so, how does it get localized? Where and by what is it made? How fast? Does it turn over? Etc.

Neuronal Cell Transplants and Cultures.- Brenner believes that while good work has been done, it has not yet been quantitative. For example, Jacobson's work on the retina: how many cells? 1,000? 10,000? 100,000?

Brenner would like to learn how many neurons in culture it takes to get functioning synapses. What does it require to get at least one criterion of change? What nutrition? Perhaps the geometrical requirements may be odd: monolayers versus impregnation of collagen sponges?

He sees the need for some analogy to the one-step growth curve of Delbrück's work on phage. He is aiming for a set of cells (an explant, a clone) that can be assembled in vitro. Then he thinks the proper tool is the EM.

Quantitative Neuroanatomy.- Brenner would like to see more quantitative human morphology. He would also like to see produced a Handbook of Wiring Diagrams, containing those circuits that have been established critically in various organisms, as by Trujillo-Cenoz in the retina and others in the cerebellum.

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